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Biological control of *Diloboderus abderus* (Coleoptera: Scarabaeidae) larvae using *Steinernema rarum* CUL (Nematoda: Steinernematidae) and *Heterorhabditis bacteriophora* SMC (Nematoda: Heterorhabditidae)

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ABSTRACT

The advance of no-till management systems in agriculture favored the increase of *Diloboderus abderus* (Sturm) populations in most of the cultivated areas in South America. Damage to wheat crops (*Triticum aestivum* Linnaeus) is caused by the larvae, commonly known as white grubs, which consume seeds and roots, weakening or killing the plants. Entomopathogenic nematodes (EPNs) are insect parasites usually used as biological control agents of larvae of Scarabaeidae species. However, a great variability in pathogenicity and virulence against different white grubs has been observed among EPN species and isolates of a single species. The aim of this work was to evaluate the pathogenicity of *Steinernema rarum* (de Doucet) CUL isolate, *Heterorhabditis bacteriophora* (Poinar) SMC isolate and their symbiotic bacteria against *D. abderus* larvae. Wheat plants were grown in plastic pots under greenhouse conditions and one larva (L1, L2 or L3) of *D. abderus* was placed in each pot and then inoculated with 1000 EPN IJs. *H. bacteriophora* caused 95 and 45% mortality to L1 and L2, respectively. No L3 mortality caused by the studied isolates was observed. Field experiments were conducted in wheat plots in 2013 and 2014. Treatments consisted of surface application of *S. rarum* or *H. bacteriophora* (2.5×10^9 IJs/ha) and chlorpyrifos; control treatments contained only distilled water. Evaluations were made at day 0, 30 and 60 after treatments by randomly selecting eight sites from each plot and determining the number of *D. abderus* L2. Larval populations were reduced in plots treated with *H. bacteriophora* and chlorpyrifos. Larval populations were reduced in plots treated with *H. bacteriophora* and chlorpyrifos. The plots treated with *S. rarum* did not show differences in the insect population with respect to the control. EPN symbiotic bacteria were injected into the hemocoel of each larval stage to determine virulence against *D. abderus*. *D. abderus* larval mortality caused by symbiotic bacteria was significantly influenced by bacterial concentration and larval stage. *H. bacteriophora* showed a greater reproductive capacity than *S. rarum*. We conclude that *H. bacteriophora* SMC have potential for use as biological control agents of *D. abderus* first and second-instar larvae.

1. Introduction

Diloboderus abderus Sturm (Coleoptera: Scarabaeidae), a polyphagous soil-dwelling pest, affects crops in Argentina, Brazil and Uruguay. Damage to wheat crops is caused by the larvae commonly known as white grub, which consume seeds and roots at 10–30 cm in soil depth, weakening or killing the plants. During severe pest attacks, the reduction in plant density generates significant yield losses (Fava and Imwinkelried, 2004).

The advance of no-till management systems in agricultural activity in the last decades has favored soil conditions for white grub

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development and has reduced larval exposure to natural enemies, with populations increasing in most cultivated areas (Silva and Salvadori, 2004). Densities of 30 larvae/m^2 can cause 70–90% production losses, with the threshold of economic damage being 5 larvae/m^2 (Aragón, 2002). This threshold is often exceeded in the Argentine Pampas region, resulting in pest control via chemical products (Laurenti et al., 2008). As this pest resides underground, the efficacy of insecticide treatments in no-till systems is below 50% (da Silva and Boss, 2002; Fava and Imwinkelried, 2004).

Entomopathogenic nematodes (EPNs) are insect parasites belonging to the genera *Heterorhabditis*, *Neosteinernema* and *Steinernema* (Nematoda: Heterorhabditidae and Steinernematidae). These organisms are used as biological control agents of crop pests of agricultural importance (Grewal et al., 2005; Lacey and Georgis, 2012). Once infective juveniles (IJs) locate the host, they penetrate through insect natural openings (mouth, anus or spiracles) or through the cuticle (Koppenhöfer et al., 2007). Once in the hemocoel, IJs release symbiotic bacteria present in their digestive tract. *Steinernema* species are associated with bacteria of the genus *Xenorhabdus*, and *Heterorhabditis* species, with *Photorhabdus* (Adams et al., 2006). Infected insects usually die of septicemia about 48 h after infection (Forst and Clarke, 2002). Nematodes then feed on the bacteria and digested tissues, enabling completion of one to three generations inside the host. Finally, IJs emerge from the host and enter the soil (Hazir et al., 2003).

EPNs have received attention as biological control agents of other white grub species (*Anomala* spp. *Popillia japonica* (Newman), *Cyclocephala* spp., *Rhizotrogus majalis* (Razoumowsky), *Phyllophaga* spp. among others) and were found to have similar control efficiency to that provided by chemical treatments (Koppenhöfer et al., 2000, 2004, 2006; Koppenhöfer and Fuzy, 2003a, 2008a; Polavarapu et al., 2007; Ansari et al., 2008; Guo et al., 2015). However, a great variability in pathogenicity and virulence against different white grubs has been observed among EPN species, and even between isolates of a single species (Grewal et al., 2002; Koppenhöfer et al., 2004, 2006).

In Argentina, the only research work focused on EPNs involving the species *D. abderus* is a survey conducted in the Pampas region, in which the species *Steinernema carpocapsae* (Weiser) and *Steinernema feltiae* (Filipjev) were naturally detected parasitizing white grub larvae (Stock, 1995). To date, however, no studies have been performed to determine the potential of EPNs as biological control agents of this pest.

The aim of this work was to evaluate the pathogenicity of *Steinernema rarum* (de Doucet) (CUL isolate), *Heterorhabditis bacteriophora* (Poinar) (SMC isolate) and their symbiotic bacteria against *D. abderus* larvae. We hypothesize that the studied EPNs are potential biological control agents of larvae of this coleopteran species.

2. Materials and methods

2.1. Study material

Isolates of *S. rarum* CUL and *H. bacteriophora* SMC, both from Santa Fe province, Argentina, were obtained from the EPN collection of the Laboratory of Zoología Agrícola de la Facultad de Ciencias Agrarias, Universidad Nacional del Litoral (FCA-UNL). They were multiplied on last-instar larvae of *Galleria mellonella* L. (Lepidoptera: Pyralidae), which were obtained from the insect collection of FCA-UNL. Nematode IJs were collected from White traps and stored at 16 °C for two weeks until use (Kaya and Stock, 1997). *D. abderus* larvae were collected from fields containing abundant populations of this insect pest in central Santa Fe province; fields had not been treated with insecticide for one year prior to insect collection.

2.2. Greenhouse experiments

The experiments were performed in greenhouses of the FCA-UNL. Plastic pots (100 mL) containing 90 g autoclaved soil (10:65:25 sand, silt and clay, respectively, and 10% relative humidity) were sown with 10 wheat seeds (variety Klein Cacique, Criadero Klein S.A.) each. After two weeks, one larva (L1, L2 or L3) of *D. abderus* was placed on the surface of each pot. The larvae that did not penetrate the soil during the following 2 h were replaced. After 48 h, 0.5 mL of an aqueous suspension containing 1000 IJs of *S. rarum* or *H. bacteriophora* was applied on the surface of each pot (Koppenhöfer et al., 2007). The control treatment received 0.5 mL of distilled water. Pots were watered regularly to keep humidity close to field capacity. Insect mortality was evaluated 7 days after inoculation with IJs. Dead larvae of *D. abderus* were dissected to confirm the presence of nematodes inside them and verify that death was caused by EPNs. Live larvae were collected and incubated in Petri dishes containing autoclaved soil at 25 °C for 7 days; those larvae whose mortality was confirmed to be caused by EPNs during that period were added to previous mortality records. For the determination of mortality rates, groups of 5 pots were considered a repetition, with four repetitions per treatment being performed in a completely randomized design.

2.3. Reproductive capacity of EPNs in larvae of D. abderus

Cadavers of *D. abderus* larvae (L1 and L2) obtained following the greenhouse procedures were removed from the plastic pots 7 days after *S. rarum* or *H. bacteriophora* IJ inoculation and placed individually in White traps (Kaya and Stock, 1997). Traps were maintained in the dark at 25 °C for 14 days to allow IJ multiplication and complete emergence. Emerged IJs were daily collected and kept at 16° C. IJs collected from each larva were suspended in 100 mL of an aqueous suspension; the number of IJs emerged from each cadaver was counted in 3 aliquots (1-mL each). Five replications were performed per insect larval stage and EPN isolate. The experiment was repeated four times (n = 20 per EPN species/insect stage).

2.4. Field experiments

The experiments were conducted in two wheat plots (typic Argiudoll soil) from the locality of Esperanza, Santa Fe province (31° 42′S, 60° 99′W), with records of high *D. abderus* population levels in the previous five years. Crops in the experimental areas had not been treated with insecticides before the experiments; after confirming the absence of EPNs (Bedding and Akhurst, 1975), 2 × 2 m plots at a minimum distance of 3 m were established. The statistical design consisted of completely randomized blocks, with four treatments and three repetitions. Experiments were performed during two consecutive years and were started on 17 June 2013 and 18 May 2014. Treatments consisted of surface application of *S. rarum* or *H. bacteriophora* at a rate of 2.5×10^9 IJs/ha (Georgis and Gaugler, 1991) and 48% chlorpyrifos at 4 L/ha (Lorsban 48E, following the manufacturer's recommendation for *D. abderus* control). Nematodes and insecticides were diluted in 2.5 L water and manually applied using a sprinkling can (Ansari et al., 2006). The control treatment consisted of an equivalent volume of distilled water. Evaluations were made at 0, 30 and 60 days after treatments. In each plot, a sampling unit was composed of eight subsamples, each consisting of randomly distributed holes of 25 × 25 cm in area and 30 cm in depth (da Silva and Boss, 2002).

The number of *D. abderus* L2 in each sampling unit was counted by direct observation (Koppenhöfer and Fuzy, 2003b) and the

results were expressed as number of larvae/m^2. Larvae were identified according to the raster pattern (Alvarado, 1980). Once counted, larvae were returned to their collection site. Environmental conditions were recorded using a data logger (Hobo Onset Corp.).

2.5. Pathogenicity of symbiotic bacteria

The bacterial isolate obtained from *S. rarum* CUL was identified as *Xenorhabdus szentirmaii* (Lengyel, Lang, Fodor, Szállás, Schumann and Stackebrandt) DSPV001N and the one obtained from *H. bacteriophora* SMC was identified as *Photorhabdus luminescens* (Thomas and Poinar) subsp. *laumondii* DSPV002N. Symbiotic bacteria were cultured in tryptic soy broth (Oxoid, England) at 28 °C for 48 h under aerobiosis and agitation at 200 rpm. *P. luminescens* and *X. szentirmaii* cultures and dilutions were measured at 600 nm (Jenway® spectrophotometer) and the number of colonies grown (CFU) on XLD plates was simultaneously determined. Absorbance and CFU parameters were evaluated using a regression analysis. To quantify the amount of bacteria obtained we used the equations $y = 0.4645 \ln(x) + 6.6619$ and $y = 0.4063 \ln(x) + 7.5013$ for *P. luminescens* and *X. szentirmaii*, respectively, where y corresponds to log10 CFU/mL and x represents the absorbance of the culture.

To determine the virulence of symbiotic bacteria against *D. abderus* larvae, bacteria were injected into the hemocoel of each larval stage. *X. szentirmaii* and *P. luminescens* were injected at the fourth abdominal segment of larvae, which were surface sterilized with 70% alcohol and washed with distilled water. Larvae were injected with 20 μL of a phosphate buffer solution (Campos-Herrera et al., 2009) containing 0, 25, 250, 2500 and 25,000 bacteria using a Hamilton micro-syringe under sterile conditions (Ansari et al., 2003). A similar volume of buffer solution containing no bacteria was injected to control larvae. After injection, larvae were individually placed in Petri dishes (9-cm diameter) containing 5 g of autoclaved sand (10% humidity) and incubated in the dark at 25 °C. Insect mortality and color change were recorded at 24, 48 and 72 h after bacterium injection. Each treatment was replicated 10 times and the experiment was completely repeated four times.

2.6. Statistical analyses

Nematode virulence (larval mortality) against *D. abderus* in the greenhouse experiments was evaluated with a two-way analysis of variance (ANOVA), with nematode species and insect larval stages as fixed effects. Reproductive capacity of EPNs was analyzed with a two-way ANOVA, with nematode species and insect larval stages as fixed effects. The number of live L2 larvae in the field trial were evaluated using a two-way ANOVA with treatment, blocks and time as fixed effects. Repeated measures were modelled using a split-plot in time approach and the Huynh-Feldt conditions were met. Virulence of symbiotic bacteria against white grubs was analyzed using groups of 10 larvae as a replicate. A four-way ANOVA test was used to analyze the effects of insect larval stage, bacterial species, bacterial rates, evaluation time and their interactions on *D. abderus* mortality.

Mortality data expressed as proportions in greenhouse experiments and pathogenicity of symbiotic bacteria were arcsine square-root transformed before analysis (non-transformed means are presented in tables and figures) to meet the requirements of normality and homogeneity of variances. Differences between means were compared using the Fisher's LSD test at the 5% probability level; all analyses were performed using INFOSTAT statistical software (Di Rienzo et al., 2016).

3. Results

3.1. Greenhouse experiments

During the experiments, mean temperature was 24.6 °C and relative humidity was 65%. Significant differences in mortality of *D. abderus* larvae were detected between the evaluated nematode species and among the insect larval stages ($F = 83.68$; $df = 1,18$; $P < 0.0001$, and $F = 125.71$; $df = 2,18$; $P < 0.0001$, respectively) (Fig. 1). A statistically significant interaction was observed between EPNs and larval stages of *D. abderus* ($F = 23.06$; $df = 2,18$; $P < 0.0001$). No larva death was recorded in control treatments nor was mortality recorded due to nematode inoculation in L3 (data not shown in Fig. 1).

3.2. Reproductive capacity of EPNs in D. abderus larvae

Heterorhabditis bacteriophora had a greater reproductive capacity on L1 and L2 of *D. abderus* than *S. rarum* ($F = 215.41$; $df = 1,76$; $P < 0.0001$) (Fig. 2). The interaction between EPNs and larval stages was statistically significant ($F = 120.37$; $df = 1,76$; $P < 0.0001$). A higher number of *H. bacteriophora* IJs emerged from L1 cadavers than from L2 ($F = 188.88$; $df = 1,38$; $P < 0.0001$). Multiplication of *S. rarum* in L1 was low, and no IJs emerged from L2.

3.3. Field experiments

In experiments conducted in 2013, mean environmental temperature and mean soil temperature (5 cm in depth) were 12.2 and 12.1 °C, respectively, relative humidity was 79% and rainfall was 9 mm. In 2014, mean environmental temperature was 13.5 °C, soil temperature was 13.8 °C, relative humidity was 84.3%, and rainfall amounted to 64 mm.

The results of experiments conducted in 2013 showed a reduction of *D. abderus* populations in plots treated with *H. bacteriophora* and chlorpyrifos ($F = 17.82$; $df = 3,16$; $P = 0.0022$) (Fig. 3). Statistical differences were detected in larval populations among the three periods evaluated, with the lowest number of insects being recorded at 60 days after the start of the experiments ($F = 91.72$; $df = 2,16$; $P < 0.0001$). A statistically significant interaction was observed between treatments and time in 2013

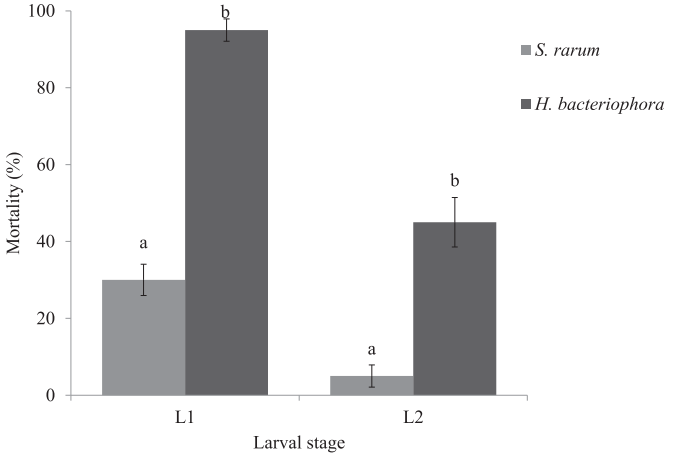

Fig. 1. Mortality of first and second larval stages (L1 and L2) of *Diloboderus abderus* caused by infective juveniles of *Steinernema rarum* CUL and *Heterorhabditis bacteriophora* SMC in greenhouse experiments. Results are expressed as mean ± standard error of the mean. Different letters indicate significant differences among treatments, according to the Fisher's LSD test ($p < 0.05$).

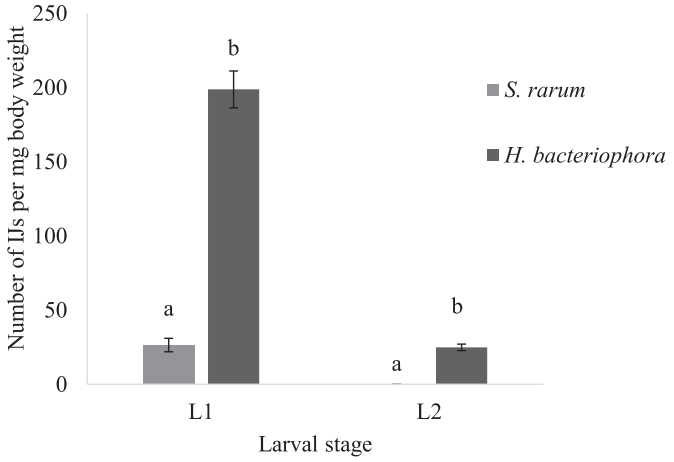

Fig. 2. Number of infective juveniles of *Steinernema rarum* CUL and *Heterorhabditis bacteriophora* SMC emerged from first-stage (L1) and second-stage (L2) larvae of *Diloboderus abderus*. Results are expressed as mean ± standard error of the mean. Different letters indicate significant differences between treatments, according to Fisher's LSD test (P < 0.05).

(F = 8.96; *df* = 6,16; *P* = 0.0002). The blocks did not differ statistically (F = 1.43; *df* = 2,16; *P* = 0.2682) and the interaction between blocks and treatments was not significant (F = 0.94; *df* = 6,16; *P* = 0.4956).

In 2014, the natural population of *D. abderus* in the experimental area was lower than in 2013. Statistical differences were detected between treatments (F = 10.54; *df* = 3,16; *P* = 0.0083) and periods evaluated (F = 32.81; *df* = 2,16; *P* < 0.0001) (Fig. 4). The interaction between treatments and time was significant (F = 3.14; *df* = 6,16; *P* = 0.0313). There were no differences between blocks (F = 1.36; *df* = 2,16; *P* = 0.2844) and the interaction between blocks and treatments was not significant (F = 1.38; *df* = 6,16; *P* = 0.2820).

The application of *H. bacteriophora* and chlorpyrifos reduced the number of insect larvae at 30 and 60 days after the start of the experiment, and their populations did not show statistical differences.

3.4. Pathogenicity of symbiotic bacteria

Analysis of mortality data by 4-way ANOVA revealed that the factors species, larval stage, bacterial concentration, evaluation time and their interactions were significant (Tables 1 and 2).

The applications of *P. luminescens* caused higher mortality of *D. abderus* larvae than that caused by *X. szentirmaii*, with L1 and L3 being the stages with highest and lowest susceptibility, respectively. The application of a higher bacterial rate of *P. luminescens* or *X. szentirmaii* was correlated with a higher larva mortality percentage. Overall, for each larval stage, mortality increased with increasing bacterial rate applied to larvae. The rate of 25,000 cells of each species caused mortality of all the insects at 72 h, regardless of the larval stage. No insect mortality was recorded in control treatments.

4. Discussion

The results of our study confirm that the isolates *S. rarum* CUL and *H. bacteriophora* SMC were pathogenic against *D. abderus* L1 and L2, but with larval stages showing different susceptibility to both EPNs. *H. bacteriophora* caused mortality of almost all the insects in the experiments involving L1, whereas *S. rarum* caused only 30% mortality. The applications of IJs to L2 showed a reduction in mortality generated by both isolates with respect to mortality observed in L1. Last-instar larvae of *D. abderus* were not susceptible to the studied nematodes.

The high susceptibility of early instars of scarab larvae to EPNs has been previously reported. Power et al. (2009) determined a higher percentage of mortality of *P. japonica* L1 and L2 than that of L3 in laboratory experiments involving infections with *H. bacteriophora* isolate GPS11. Field experiments conducted with the same isolate demonstrated the efficacy of nematode applications in the control L1 and L2 of *P. japonica*. The susceptibility of *Anomala orientalis* (Waterhouse) to *H. bacteriophora* isolate TF was also lower in L3 than in L2 (Koppenhöfer and Fuzy, 2004). Lee et al. (2002) reported that *Heterorhabditis* sp. Gyeongsan caused 100% mortality in L2 of *A. orientalis*, and only 38% in L3 larvae. However, L3 scarabs were found to be more susceptible than earlier stages

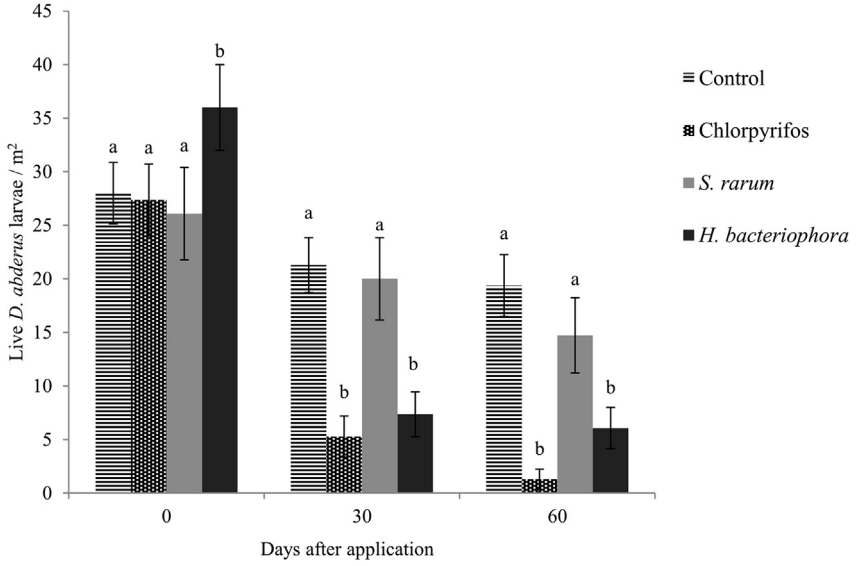

Fig. 3. Number of *Diloboderus abderus* L2/m^2 in control plots, as well as in plots treated with chlorpyrifos and with infective juveniles of *Steinernema rarum* CUL or *Heterorhabditis bacteriophora* SMC in field experiments conducted in 2013. Results are expressed as mean ± standard error of the mean. Different letters indicate significant differences between treatments in each evaluation period, according to the Fisher's LSD test (P < 0.05).

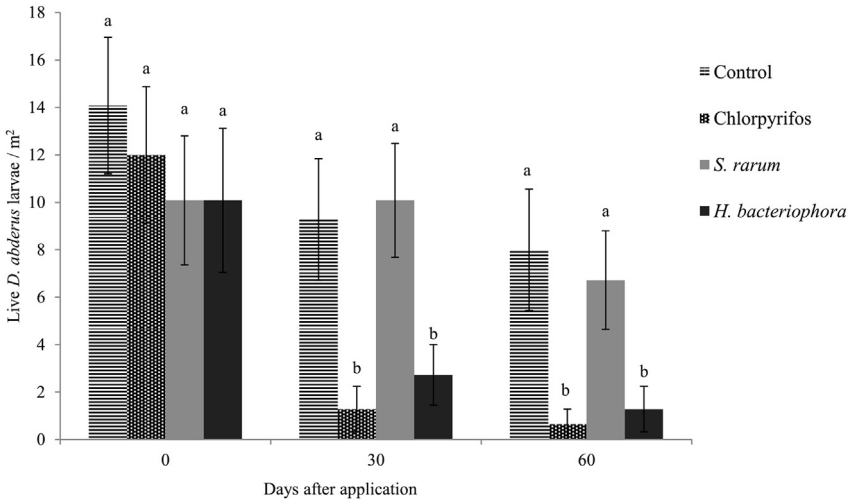

Fig. 4. Number of *Diloboderus abderus* L2/m^2 in control plots, as well as in plots treated with chlorpyrifos and with infective juveniles of *Steinernema rarum* CUL or *Heterorhabditis bacteriophora* SMC in field experiments conducted in 2014. Results are expressed as mean ± standard error of the mean. Different letters indicate significant differences between treatments in each evaluation period, according to the Fisher's LSD test ($P < 0.05$).

Table 1
Pathogenicity of different concentrations of *Xenorhabdus szentirmaii* DSPV001N and *Photorhabdus luminescens* DSPV002N injected in the hemocoel of the three larval stages of *Diloboderus abderus*.

Bacterial concentration	Mortality percentage of *D. abderus* larvae (mean ± SEM)								
	L1			L2			L3		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
X. szentirmaii DSPV001N									
0	0±0a	0±0a	0±0a	0±0a	0±0a	0±0a	0±0a	0±0a	0±0a
25	0±0a	7.5 ± 4.79b	22.5 ± 6.29de	0±0a	0±0a	0±0a	0±0a	0±0a	0±0a
250	0±0a	15 ± 2.89cd	27.5 ± 4.79e	0±0a	0±0a	12.5 ± 2.5c	0±0a	0±0a	0±0a
2500	0±0a	20 ± 4.08cde	45 ± 6.45f	0±0a	37.5 ± 4.79f	42.5 ± 7.5f	0±0a	7.5 ± 4.79b	20 ± 4.08cde
25,000	65 ± 9.57gh	100±0j	100±0j	25 ± 6.45e	100±0j	100±0j	0±0a	100±0j	100±0j
P. luminescens DSPV002N									
0	0±0a	0±0a	0±0a	0±0a	0±0a	0±0a	0±0a	0±0a	0±0a
25	0±0a	100±0j	100±0j	0±0a	0±0a	0±0a	0±0a	0±0a	0±0a
250	40 ± 7.07f	100±0j	100±0j	40 ± 9.13f	72.5 ± 7.5 h	100±0j	0±0a	62.5 ± 4.79g	85 ± 6.45i
2500	100±0j	100±0j	100±0j	100±0j	100±0j	100±0j	2.5 ± 2.5a	100±0j	100±0j
25,000	100±0j	100±0j	100±0j	100±0j	100±0j	100±0j	100±0j	100±0j	100±0j

*Means followed by the same letter are not significantly different from each other according to Fisher's LSD Test ($p < 0.05$).

Table 2
Results of four-way ANOVA examining the effects of insect larval stage, bacterial species, bacterial rates and evaluation time on *D. abderus* larval mortality.

Effect	*df*	F value	*P*
Species (S)	1	4434.06	<0.0001
Stages (St)	2	554.61	<0.0001
Rates (R)	4	4079.72	<0.0001
Evaluation time (T)	2	1128.22	<0.0001
S x St	2	66.72	<0.0001
S x R	4	645.54	<0.0001
S x T	2	2.11	0.1229
St x R	8	120.54	<0.0001
St x T	4	31.67	<0.0001
R x T	8	91.58	<0.0001
S x St x R	8	67.28	<0.0001
S x St x T	4	32.71	<0.0001
St x R x T	16	58.43	<0.0001
S x R x T	8	168.34	<0.0001
S x St x R x T	16	55.00	<0.0001

(Ansari et al., 2008; Smits et al., 1994). These data show the need to determine the pathogenicity of each EPN isolate against a given scarab species.

To date, little is known about the behavior of *S. rarum* in the presence of white grubs. The species was found to cause only 2% mortality in L3 of *Cyclocephala hirta* (LeConte) (Koppenhöfer and Kaya, 1999). There are no previous data on the infectivity of this EPN in different larval stages of white grubs. Here, results of the field experiments indicated that the application of *H. bacteriophora* IJs reduced 68 and 84% of the white grub population in 2013 and 2014, respectively. By contrast, plots treated with *S. rarum* did not show a reduction in the number of pest larvae with respect to control treatments.

Mortality levels obtained using *H. bacteriophora* can be attributed to the cruise search strategy of this nematode species, which allows it to actively move through the soil in search of its hosts (Campbell and Gaugler, 1997). Although *D. abderus* larvae dig galleries in the soil, they often stay immobilized for long periods in small chambers they build between 10 and 30 cm deep in the soil (Morelli, 1997). The cruise foraging strategy of *H. bacteriophora* allows this nematode to encounter pest larvae and even infect them inside the chambers, where the insect-soil surface contact is reduced. The capacity of this EPN to infect the insect pest inside the

chambers was confirmed in our research when we evaluated the experimental units of the greenhouse experiments.

The differences in virulence observed in the studied EPN species against the insect larval stages may be attributed to the capacity of IJ for cuticle penetration as well as to the insect immune response. Regarding cuticle penetration, Koppenhöfer et al. (2007) and Wang and Gaugler (1998) demonstrated that IJs of *H. bacteriophora* have excellent ability to penetrate through the cuticle of scarabs. This ability becomes especially important because spiracle penetration is hindered by the presence of protective "sieve plates" in white grubs (Forschler and Gardner, 1991) and the high defecation rate of this pest reduces infection via the anus (Hazir et al., 2003). The high cuticular sclerotization of the *D. abderus* third stage, however, would limit penetration by IJs. Furthermore, the insect's immune response can affect the pathogen and is host- and EPN isolate-specific (An et al., 2012; Li et al., 2007). Li et al. (2007) confirmed that, when penetrating larvae of *Exomala orientalis* (Waterhouse), *P. japonica* and *Cyclocephala borealis* (Arrow), IJs of *H. bacteriophora* HP88 are melanized and can hardly escape insect reaction. Despite the defenses of white grubs to the attack of EPNs, some nematodes succeed in avoiding the insect defenses and can release the symbiotic bacteria, causing the host death (Alvandi et al., 2014). An et al. (2012) stated that differences between the virulence of *H. bacteriophora* inbred lines against *P. japonica* and *C. borealis* were related to the ability of nematodes to break down encapsulation caused by the immune response of larvae rather than to the proportion of IJs penetrating the host.

The application of *S. rarum* IJs in field experiments produced a lower reduction of the larval population of *D. abderus* than *H. bacteriophora*. This nematode species is adapted to moisture changes commonly occurring in the upper soil centimeters, and has an intermediate host searching strategy between "ambusher" and "cruiser" (Koppenhöfer and Kaya, 1999). This behavior might affect the search of larvae at greater depths in the soil profile, especially in loamy soils as those used in this research. Furthermore, Koppenhöfer and Kaya (1999) determined that *S. rarum* shows preference for parasitizing lepidopteran hosts.

The most numerous nematode offspring was obtained using the isolate *H. bacteriophora* SMC in *D. abderus* L1. *S. rarum* did not multiply on L2 and emergence of *H. bacteriophora* IJs was limited. Koppenhöfer and Fuzy (2008b) estimated offspring of more than 11,000 IJs when infecting L3 of other four scarab species with *H. bacteriophora* GPS11. The studied isolates did not show to have a good multiplication capacity on the insect. The only work that studied the progeny of the isolates used in this work (Del Valle et al., 2016) found that *S. rarum* produced a higher number of IJs on larvae of the coleopteran *Alphitobius diaperinus* (Panzer) than *H. bacteriophora*, indicating the influence of the host species on the reproductive capacity of the studied isolates.

Symbiotic bacteria associated with EPNs were pathogenic to the three larval stages of *D. abderus*, with *P. luminescens* DSPV002N being more virulent than *X. szentirmaii* DSPV001N. The high pathogenicity of *P. luminescens* against scarabids was confirmed by Ansari et al. (2003) using larvae of *Hoplia philanthus* (Fuessley). Our results indicate that IJ penetration into the hemocoel of the larva can be a limiting factor to the pathogenic process in L3, as observed by An and Grewal (2007) in larvae of *R. majalis*.

This is the first research work studying the pathogenicity of EPNs and symbiotic bacteria against larvae of *D. abderus*. We conclude that IJs of *H. bacteriophora* SMC have potential for use as biological control agents of first- and second-instar larvae of the coleopteran. In future research works we intend to study persistence of *H. bacteriophora* SMC in Pampas soils and the nematode effect on pest population levels over time.

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